ANATOMY OF THE PALM RHAPIS EXCELSA, IV. VASCULAR DEVELOPMENT IN APEX OF VEGETATIVE AËRIAL AXIS AND RHIZOME[1]

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PREVIOUS ARTICLES IN THIS SERIES have analyzed quantitatively the course of vascular bundles in the vegetative aërial stem and in the rhizome of *Rhapis excelsa* (Zimmermann & Tomlinson, 1965; Tomlinson & Zimmermann, 1966a). The present article extends our descriptive analysis of *Rhapis* to the apex of both aërial and underground axes, tracing vascular strands, in their earliest recognizable condition as procambial strands.[2] From this we can make certain deductions about the way in which the vascular system develops. No attempt is made to describe the development of phloem and xylem tissues within the procambial strands, this important aspect being reserved for a future article.

As Esau (1965, p. 33) has pointed out, information on leaf trace relationships in monocotyledons, especially the perennial types, is incomplete; the apparent complexity of these plants has inhibited their investigation. Nevertheless our analysis of *Rhapis* has shown that the basic plan is quite simple, only the overall large number of bundles obscures this.

Despite the rather artificial attempts by earlier anatomists to classify monocotyledonous vascular systems (e.g., by Falkenberg, 1876), only two main groups need be considered (Priestley & Scott, 1937), namely those with a nodal plexus of vascular tissue, exemplified by the grasses, and those without nodal plexi, exemplified by the palms. The former have been studied quite extensively (Kumazawa, 1961; Scott and Priestley, 1925; Sharman, 1942). However, in the non-nodal type of monocotyledons the only study which continuously relates development of the vascular strands to their distribution in the mature stem is that by Priestley *et al.* (1935) in *Alstroemeria*, an analysis which is worthy of wide attention. A notable observation of these workers is that vascular bundles are recognizable as acropetally growing strands in the developing stem long before the leaf which they ultimately supply is produced. *Alstroemeria* is, however, a relatively diminutive monocotyledon and its vascular system is much less elaborate than that of even the smallest palm.

Because of the greater bulk and complexity of palms, workers have been content to describe development in general terms. The earliest literature

[1] A further contribution towards a continuing study of the anatomy of the palm stem by one of us (P.B.T.) supported by N.S.F. Grant GB 2991.

[2] The terminology used in this paper, as well as in the others of this series, is a purely descriptive anatomical one (see the statement in Zimmermann & Tomlinson, 1965, p. 167 top).

discusses mostly the hypothetical "endogenous" growth of vascular bundles and is now largely of historical significance. Branner (1884) summarizes this history in his introductory pages. Branner's own factual contribution is noteworthy because he grasped the principle governing the distribution of vascular bundles in the mature palm stem, but he obscured it in a developmental discussion which seems not to be based on observation. Falkenberg's (1876) developmental account is also theoretical. His illustration of the palm is at best incomplete and, as we have shown, has been more a source of error than information for later writers (Tomlinson & Zimmermann, 1966b). Baranetzky's (1897) account of vascular bundle development in monocotyledons describes simply a centrifugal or centripetal, sometimes mixed centrifugal and centripetal, direction of development but adds nothing to our knowledge of the longitudinal course of differentiation. More recently Ball (1941) has verified the "centrifugal" development of vascular bundles recorded by Baranetzky in palms. Ball and other writers on the shoot apex of palms (Chouard, 1936; Eckardt, 1941; Helm, 1936) have simply described and illustrated the general topography of the meristematic regions in palms, showing that thickening growth of the stem is not a direct result of the shoot apical meristem proper but is associated with a primary thickening meristem developed below the expanding leaf primordia.[3] Otherwise no attempt seems to have been made to follow the distribution of leaf traces in the meristematic crown, although it is clear that an understanding of development is impossible without knowledge of the course of vascular bundles.

In *Rhapis*, as in other small palms, activity of the primary thickening meristem is such that the overall outline of the meristematic crown is a shallow cone rather than a bowl. This is fortunate, because analysis is facilitated when vascular bundles can be followed continuously in one direction through serial sections. The crown is also of a size suitable for handling by orthodox microtechniques. In addition, our previous quantitative analysis of the mature *Rhapis* stem has provided the information about vascular organization which, as Esau (1965) emphasizes, is an essential prerequisite for developmental understanding. For convenience this vascular organization in mature stems is very briefly outlined below, although the reader is referred to the first two papers of this series for details (Zimmermann & Tomlinson, 1965; Tomlinson & Zimmermann, 1966a). Familiarity with the facts in these two articles will greatly facilitate the understanding of the present paper.

In *Rhapis* all bundles of the central cylinder behave essentially alike. Each axially running vascular bundle is linked, at regular intervals, to leaves via a branch, the leaf trace. We have called the distance between leaf contacts of a given bundle the "leaf-contact distance" or "leaf-contact interval." This distance can be measured in number of internodes (cf. Zimmermann & Tomlinson, 1965, p. 169, *Fig. 3*). The departing leaf trace

[3] Distinction must be made between the shoot apex proper, which in palms produces only leaf primordia, and the meristematic region of the whole apex. Subsequently, the former will be referred to as "apex", the latter as the "crown."

shows the familiar outward curve originally recognized by von Mohl (1824) as characteristic for all palms. Each leaf is supplied by relatively few major bundles, but by a larger number of intermediate and minor bundles. Major leaf traces originate from the more central bundles so their outward curvature is most pronounced. Major bundles also give off leaf traces at the longest intervals (about 15 internodes). Intermediate and minor bundles are progressively more restricted to the periphery of the central cylinder and consequently the outward curvature of their leaf trace branches is less pronounced. In addition the leaf-contact distance is progressively shorter for bundles farther towards the periphery. Each continuing bundle (vertical bundle) above the level of departure of the leaf trace initially follows the outward curve of the leaf trace to the crowded periphery of the central cylinder, whereupon it turns erect and then gradually approaches the stem center again to give off a further leaf trace at some higher level (cf. Zimmermann & Tomlinson, 1965, p. 169, *Fig. 3*). During this gradual inward movement the bundle describes a shallow helix in the direction of the phyllotactic spiral. In the rhizome bundles deviate less from the axial direction and the helical path is scarcely evident (cf. Tomlinson & Zimmermann, 1966a, p. 254, *Fig. 4* below). In addition, there is greater irregularity of leaf-contact distance and bundle continuity.

In both axes lateral continuity between different bundles is achieved by short bridges between outgoing leaf traces and nearby vertical bundles. Cortical bundles occur in both types of axes as anastomosing strands continuous from the leaves, independent of the vascular system in the central cylinder, and ultimately ending blindly below.

PREPARATION OF MATERIAL

Vegetative stem and rhizome apices of *Rhapis excelsa*, together with enveloping leaf bases, were isolated from plants cultivated at Fairchild Tropical Garden and fixed in FAA. During dissection a record was kept of the state of development of the youngest exposed leaves in such a way that the corresponding leaves could be identified in serial sections. Stem apices and surrounding leaves thus prepared were cylindrical blocks of the order of 1–1½ × 2–3 cm. Blocks were desilicified in hydrofluoric acid, dehydrated and embedded in paraffin wax by routine procedures and sectioned serially at thicknesses from 8 to 15 μ. Shrinkage of material was inevitable but did not interfere with subsequent analysis. Sections were stained either in safranin and Delafield's haematoxylin on the one hand, or in erythrosin and toluidin blue on the other. Most observations were made on serial transverse sections but longitudinal series were also used for comparison. The bottom slides of the series of sections show all the anatomical features of the mature axis except that tissues are immature, e.g., metaxylem elements and fibers have not yet developed secondary walls.

GENERAL TOPOGRAPHY OF THE CROWN

For the sake of clarity we shall now introduce the reader to the general topography of the crown. This actually represents part of the results of the present study; but by giving this short initial description it will be easier for the reader to understand the next chapter of the paper describing the method of serial-section analysis wherein we break newer ground.

Aërial stem (FIGS. 1, 2, 6, and 7). We have not investigated the shoot apical meristem proper. Reference may be made to Ball's (1941) work on this subject in those species he examined. In palms this apical meristem functions largely as a "leaf-initiating" meristem. In *Rhapis* it includes no procambial tissue, i.e., no vascular strands can be discerned above the youngest primordium. The overall outline of the crown of *Rhapis* is a shallow cone, without the bowl-shaped depression of larger palms. This difference between smaller and larger palms is related to the activity of the primary thickening meristem and is not a fundamental one.

Leaves in *Rhapis* are arranged in a 2/5 phyllotactic spiral (FIG. 1). Each leaf has a closed tubular base tightly enclosing younger primordia, but somewhat thickened on the dorsal side. This dorsiventral difference in thickening is more pronounced distally in each leaf and is most easily seen in successively younger leaves cut at the same level, as towards the center of FIG. 1. FIG. 1 is drawn from a section cut too high to show the circular attachment of primordia 2–5. This leaf-base topography is the result of leaf growth by a pronounced basal meristem after the establishment of an encircling attachment. Development of this encircling attachment in the young primordia must be rapid because in the sections examined it is only incomplete in the youngest primordium (P1).

Procambial strands are evident even in the youngest primordium included in FIG. 1, their further rapid increase is indicated in the counts for total numbers of strands in successively older leaves plotted in FIG. 4. Immediately below the apical meristem proper, and continuous with it, is a dome-shaped or umbrella-like mass of meristematic cells. This undifferentiated meristematic "cap" (subsequently referred to simply as the cap) is pierced by leaf traces entering the youngest primordia. The cap is more or less easily visible on single sections of the rhizome crown, but it is much less easily recognizable in the crown of the aërial stem, except if one runs rapidly through transverse section series with the motion-picture analyzer.

Towards the lower border of the cap individual procambial strands are distinguishable from vacuolating cells. These strands are the distal extremities of the vertical bundles which, when traced upwards from below, end blindly in the cap. This cap-like meristem therefore is continuous with the crowded periphery of the central cylinder of the mature stem. The more central stem tissue, immediately below the shoot apex and enclosed by the cap, contains procambial strands already linked to leaves via the leaf traces which penetrate the cap.

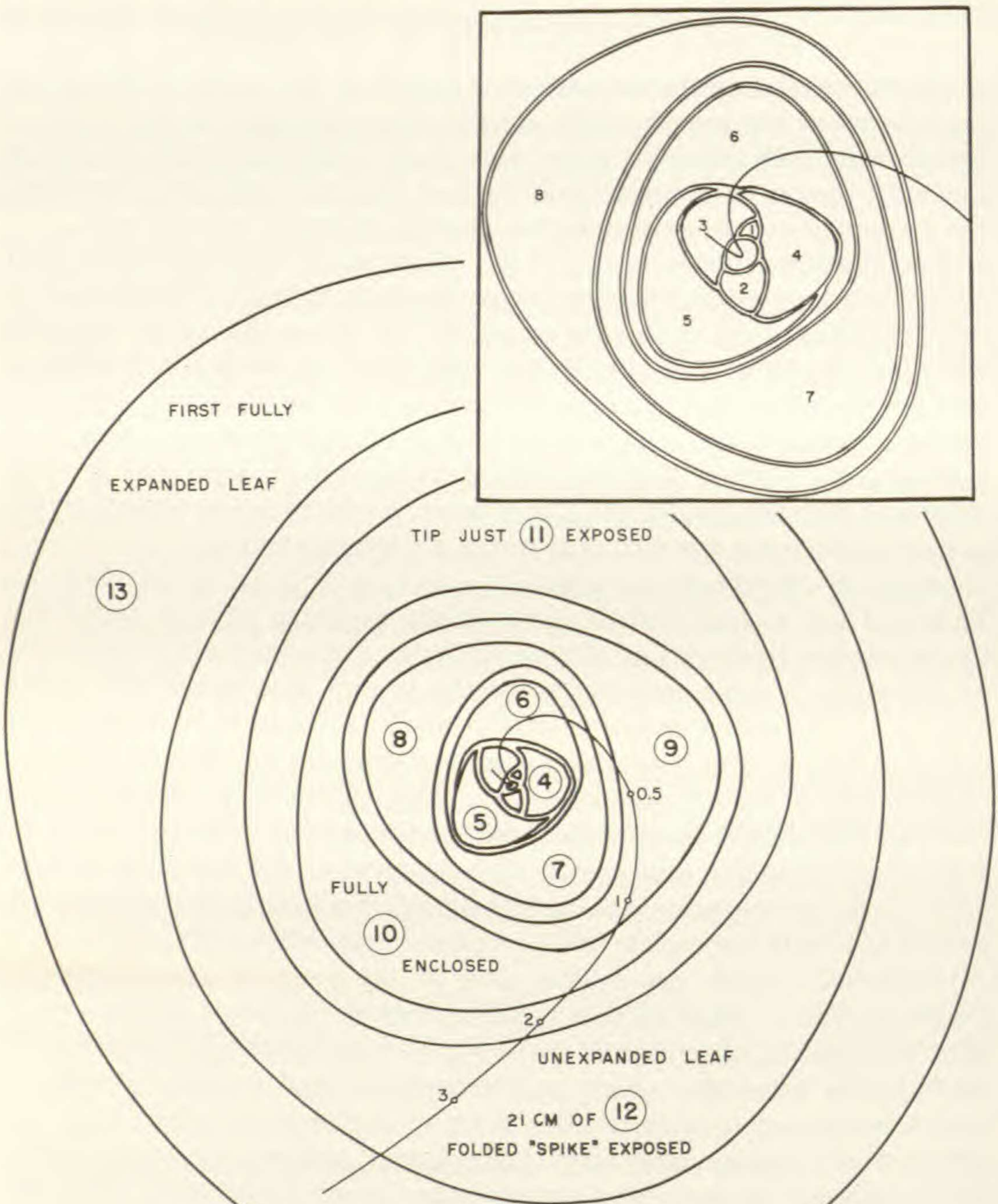


FIG. 1. *Rhapis excelsa*. Transverse section through the crown of the vegetative aërial axis. Leaf primordia are numbered from within, number 1 (P1) is the youngest that can be discerned, P14 is omitted. P1 is the only primordium which does not completely encircle the stem at its attachment, P2–P5 are cut above their encircling bases. This crown is the same as the one shown in the diagrammatic longitudinal section on FIG. 2. The spiral indicates the path of a dorsal major leaf trace from P3 (note the sharp turn below the apex). The numbers along the spiral indicate millimeters below the apex. INSET: central portion of section at higher magnification.

It must be emphasized at this point that the cap is *not* identical with the primary thickening meristem. The primary thickening meristem must be located *under* (i.e. inside) the cap. In neither of the crowns can the primary thickening meristem be structurally recognized in single sections because cell divisions are not uniform in any one direction. Towards the base of the crown, however, at the level of insertion of recently expanded leaves, ground meristem cells divide in a predominantly transverse plane, producing the vertical files of cells which indicate uniform elongation of the axis without distinction between nodal and internodal regions (FIG. 7).

Procambial strands in longitudinal section are strikingly distinct from the surrounding ground tissue as fascicles of cells with coincident end walls. The nuclei of each tier of cells at the same level produces a "tiger-tail" effect in longitudinal view. This indicates the origin of procambial strands from undifferentiated meristem by numerous longitudinal divisions in series of meristematic cells, the common end wall of each tier indicating the limits of each parent meristematic cell. This coincidence of end walls persists in the vascular tissues of mature vascular bundles (Tomlinson, 1961. p. 58). In transverse section procambial strands are less conspicuous, except where the sections include the nuclei. Otherwise they are distinguished by their narrow cells with somewhat densely staining cytoplasm. Limits of procambial strands are not sharp in the more meristematic regions where they become progressively more difficult to recognize.

Rhizome (FIGS. 3, 8, and 9). The apical region of the rhizome (FIG. 8) is similar to that of the aërial stem in all essential details, except for being smaller (cf. FIGS. 2 and 3). The 2/5 phyllotactic spiral is less obvious because the dorsiventral asymmetry of the leaves is less marked. The meristematic cap at the lower edge of which the vertical bundles terminate is more clearly visible in the rhizome than in the aërial stem (FIG. 9).

Helical path of bundles. The helical path of the procambial strands in the uncrowded center of the crown is not evident in any single section. Only a short length of any one bundle is included in single sections because of the conical shape of the crown. However, the spiral path can be demonstrated by plots of bundles. As an example, a major trace of P3 is given in FIG. 1. The trace is projected vertically into a transverse plane, the numbers along its path representing the vertical distance below the apex in millimeters.

In flat or bowl-shaped crowns of larger palms the overall spiral path of a number of bundles can be included within a single section. This is shown, for example, in *Chrysalidocarpus lutescens* (FIG. 5). This demonstration of the helix as an inherent feature of the crown in palms is historically significant because it finally settles an old controversy. Meneghini (1836) suggested that the helix is a mechanical consequence of torsions set up in the palm stem as it develops. This suggestion was debated at some length by early authors such as von Mohl (1849) but without examination of developing palm stems. Clearly the initiation of vascular bundles along a spiral pathway requires a physiological explanation, not a mechanical one.

METHOD OF SERIAL-SECTION ANALYSIS

This chapter describes how the serial transverse sections were analyzed. and how FIGS. 2 and 3 were constructed. These two figures contain most of the information obtained during the present study; they form the basis for subsequent discussion of development.

Two aërial and one rhizome crown were analyzed in full quantitative detail. Both aërial crowns showed an almost identical course of vascular strands, they will therefore not be discussed separately.

Initially the sections were surveyed so that the general topography of the crown could be established. Diameter measurements of the axis at each leaf insertion (in its dorsiventral plane) were plotted against section number. The resulting diagram shows a radial longitudinal section of the apex, *but with all the leaves in the same plane* (FIGS. 2 and 3). The vertical scale of these figures indicates the axial dimensions (i.e., the thickness of the sections when stacked up) and the horizontal scale is the radial distance of the plotted strands from the stem center. Measurements of peripheral strands were made from the strand to the stem periphery, more central strands were measured from the strand to the stem center, which was established by eye and marked with a spot of India ink on the cover glass. This visual determination of the center is of ample accuracy because the transverse sections are far from exactly circular. It did mean that plotted positions do not fall precisely on the smooth curves drawn in FIGS. 2 and 3; to this extent these plots are made diagrammatic. Of course, bundles do run along a more or less smooth path; if plotted curves come out a little irregular it is because there is no fixed point of reference in the axis.

As a given vascular strand was followed, its distance from the stem center was measured about once every 300 μ and its position was then plotted on the diagram. The way in which continuous recognition of the same strand was achieved is described below. The largest major bundle from each leaf was followed in turn, from the leaf insertion to the base of the crown (the lowest section in the series). Plotting started with the oldest available leaf (P14) and continued with each progressively younger leaf. Each bundle having been followed in the downward direction as a leaf trace, plotting was then continued in the reverse direction but now along its diverging vertical bundle, as far towards the shoot apex as the strand could be discerned. For successively younger leaves this operation became increasingly difficult because their traces are correspondingly less well differentiated.

FIGS. 2 and 3 show, therefore (as solid lines), the course of the largest major leaf trace which enters the axis from the dorsal side of the leaf. A minor and a cortical bundle from P11 are also indicated in FIG. 2. It must be emphasized again that these figures are not drawings of actual longitudinal sections because all dorsal sides of the leaves have been rotated into a single radial plane. Furthermore, the spiral path of the strands is entirely ignored. This spiral path is shown for a major bundle from P3 in FIG. 1.

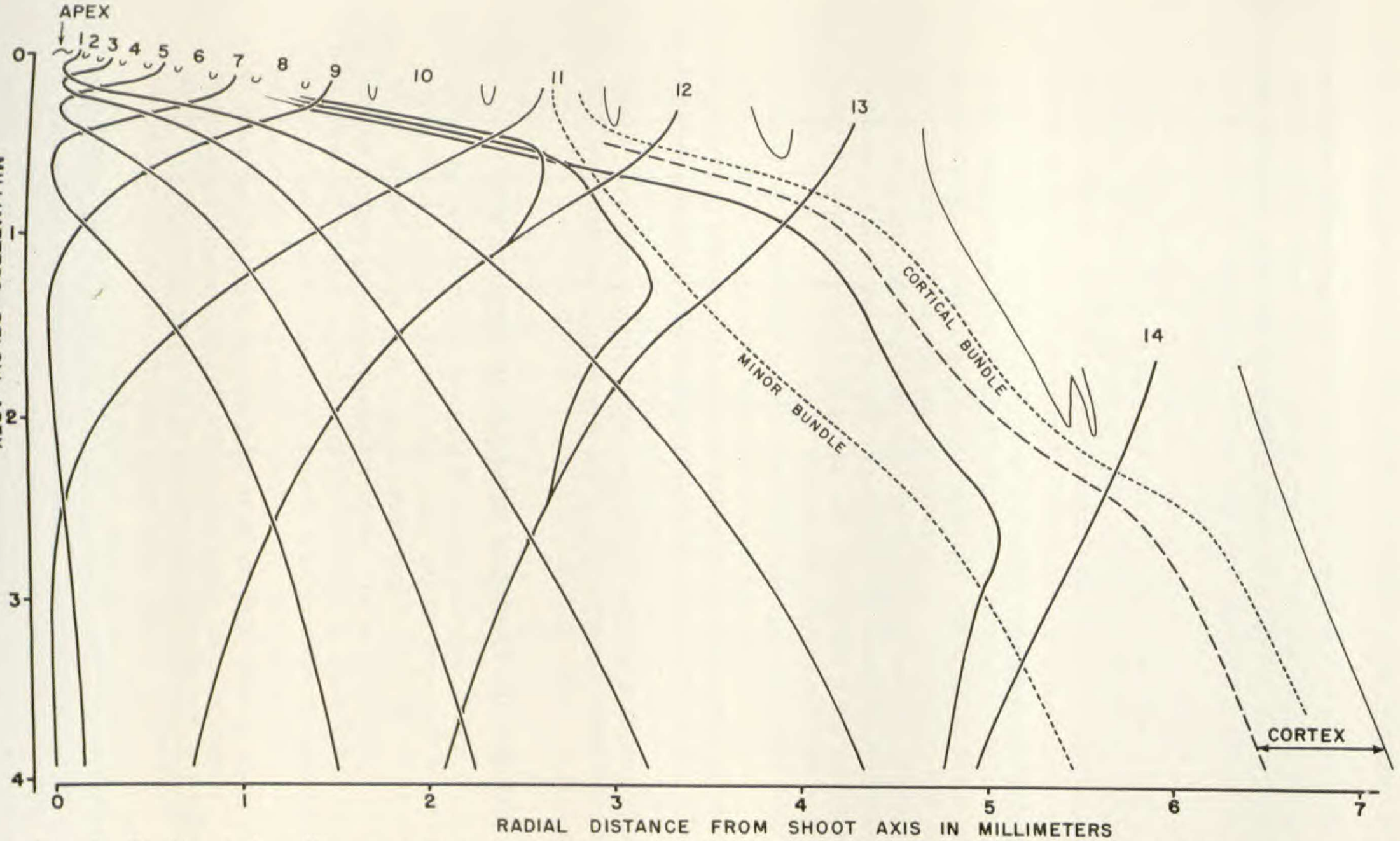


FIG. 2. *Rhapis excelsa*. Diagrammatic radial longitudinal section through the crown of the aërial vegetative axis based on measurements as described in the text. This is not a drawing of a single longitudinal section. All dorsal sides of leaf insertions are rotated into a single radial plane so that major leaf traces can be compared. The spiral path of the bundles is ignored. Leaf numbering as in FIG. 1. All solid lines are major bundles. A minor and a cortical (fibrous) bundle of P11 are included for comparison (dotted lines). Broken line separates cortex from central cylinder. Traces to P2, P4, P6, P8, and P10 are omitted for the sake of clarity.

projected vertically onto a tranverse plane. The same trace is shown in FIG. 2.

The reliability of these plots depends on the certainty with which a single bundle can be followed. Anyone who carefully looks at the photomicrographs in this article will realize that it is not easy to follow an individual bundle throughout a series of sections, especially if he considers that the stem contains about a thousand central vascular, and another two thousand five hundred cortical bundles. Therefore, during the plotting a motion picture was prepared with the drawing method outlined earlier (Zimmermann & Tomlinson, 1965). This procedure provided the physical discipline needed to ensure that continuity of a vascular strand was not lost. The problem of loss of continuity became increasingly severe in the vascular strands associated with the youngest primordia. Here the procambial strands are narrow, poorly differentiated and make sharp turns near the center of the stem. However, it was found that in critical areas (the top half millimeter), by making two superimposed drawings in contrasting colors at two magnifications for each section, the procambial strands could be followed with certainty. The drawing at low magnification served for quick orientation, the one at high magnification for identification of the strand under observation.

The most important data thus obtained are the resulting plots (FIGS. 2 and 3). Nevertheless, the motion pictures were quite useful to verify continuity and to allow study of incidental anatomical features. Moreover, it demonstrates in a few minutes the discoveries resulting from several weeks of hard work. In the motion picture of the rhizome crown, for example, the cap can be seen as a closing diaphragm which "sweeps away" all vertical bundles; a dynamic demonstration of the way vertical bundles "fuse" into the cap.

COURSE OF VASCULAR BUNDLES IN THE CROWNS

FIGURES 2 and 3 show the course of vascular traces in the top 3 to 4 millimeters of the crown of the aërial axis and the rhizome respectively. They are all dorsal major traces except those clearly labelled in the figure. These two figures show a very complex three-dimensional system reduced to a single radial plane, as described in the previous chapter. In order to reconstruct the three-dimensional system, the reader has to go through the following mental exercise. All points of entry of dorsal major bundles from leaf base to stem have to be rotated around the stem axis back to their five respective radial plans so that the 2/5 phyllotactic requirement is fullfilled (i.e., P1, P6, P11, etc. belong to the same orthostichy). Then, looking along the stem axis, one has to twist the five radial planes into the spiral shape shown for the trace to P3 in FIG. 1.

A comparison of FIGS. 2 and 3 with the diagrams of the adult axes, shown in previous papers (cf. Tomlinson & Zimmermann, 1966a, *Fig. 4*, below) shows clearly that the vascular pattern of the adult axes is laid down in the crown *before primary thickening growth occurs*. In the crown, leaf traces run axially and vertical bundles run radially. Subsequent

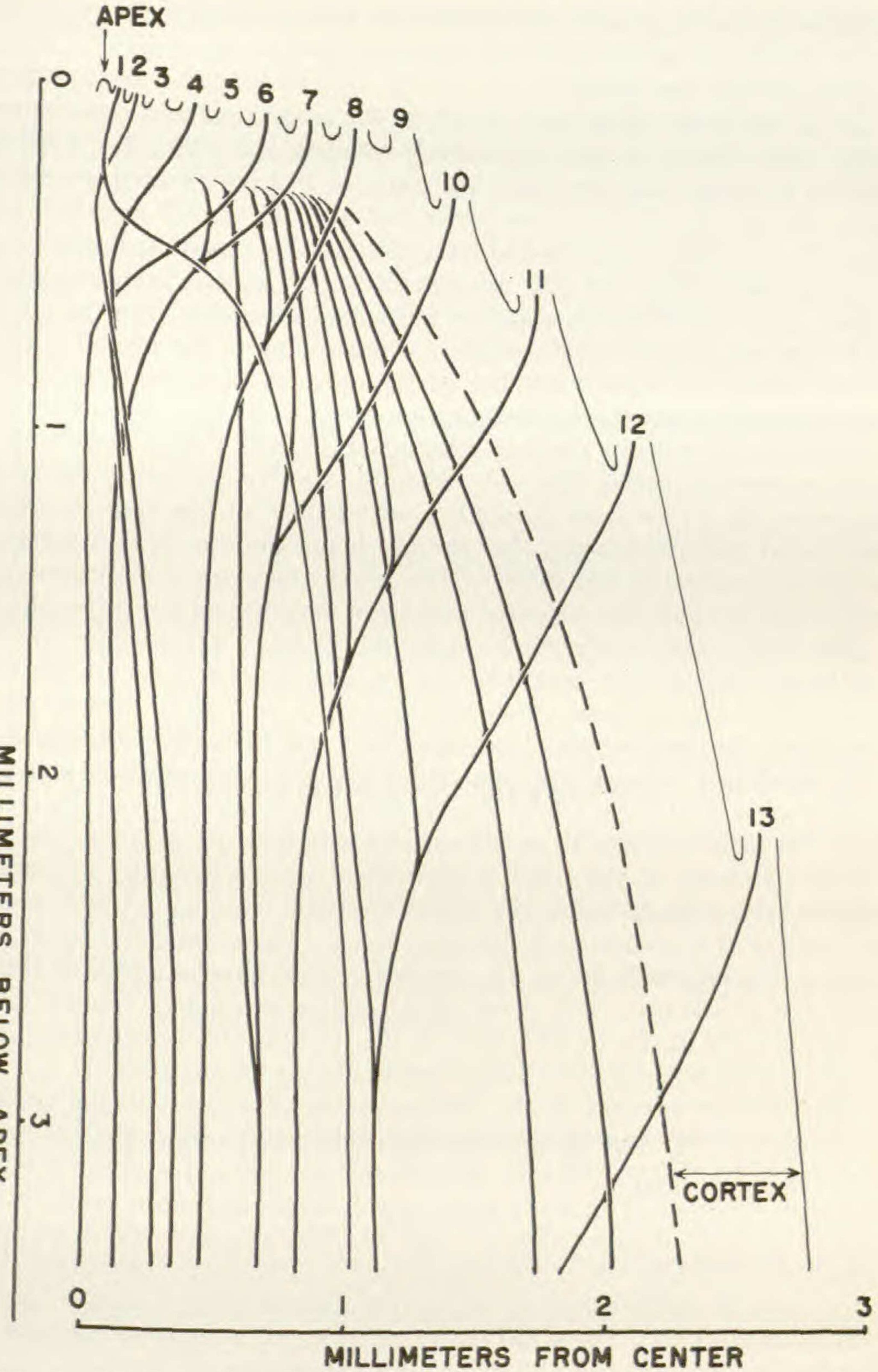


FIG. 3. *Rhapis excelsa*. Diagrammatic radial section through the crown of the rhizome based on measurements as described in the text. Like FIG. 2, this is not a drawing of an actual longitudinal section; all dorsal sides of leaf insertions are rotated to a single radial plane so that successive stages of development of major leaf traces can be compared. All solid lines are major bundles. Broken line separates cortex from central cylinder. Traces to P3, P5, and P9 are omitted for the sake of clarity.

primary thickening growth reorientates the system through 90°, with the result that in the mature stem leaf traces run more or less radially, vertical bundles more or less axially.

Let us now look a little more closely at the aërial stem and examine the major trace system of each successively younger leaf (FIG. 2). Vertical bundles diverging from the major leaf traces to P14 are released somewhat below the bottom of the section series, but are still readily identified because they initially follow the leaf trace closely in its centrifugal path. The major traces to P13 and P12 are complete, but located proportionately higher. No vertical bundle could be recognized diverging from the traces to P11 or any younger primordium. Corresponding to the overall outline of the crown, all vertical bundles of the crowded peripheral region are approximately horizontal at the insertion of P13. In this region they are conspicuous as radially running procambial strands ("tiger tails") in a single transverse section (FIG. 6). About 1/4 millimeter below the apex, approximately at the level of insertion of P8 they all fuse into an undifferentiated meristematic cap (but the cap is not uniform, it is penetrated axially by procambial leaf traces). The cap is, therefore, the meristematic region out of which the congested peripheral vertical bundles differentiate.

Leaf traces, even those which supply the youngest primordium, are all continuous; below they consist of the vertical bundle, above of the leaf trace. The ultimate basal continuity cannot, of course, be demonstrated directly in the short length of axis examined, but since the basal region is fully developed (though not fully differentiated), extrapolation into the mature stem is obvious. With a leaf-contact distance of about 15 internodes for major bundles P1 would connect with P16, P2 with P17, etc.

The curvature of the trace is established by reorientation of central bundles, immediately below the apical meristem, through a right angle; the method of reorientation is described below. These distal leaf traces are more or less perpendicular to the crowded vertical bundles and they pierce the meristematic cap. This development of two procambial systems more or less at right angles to each other is the fundamental feature which accounts for the final configuration of bundles in the mature stem.

The vascular anatomy of the rhizome crown (FIG. 3) is similar to that of the aërial stem, with quantitative differences which reflect distinguishing features of the mature rhizome. Inward and outward curvature of bundles is less pronounced. The meristematic cap is larger and more easily recognizable in single sections (FIGS. 8 and 9). The youngest leaf trace with attached vertical bundle is that supplying P7. The greater plasticity (i.e., irregularity in construction) of the mature rhizome trace system is further verified in the crown where leaf traces may give off more than one vertical bundle (as the traces to P11 and P12, FIG. 3) or none.

DEVELOPMENTAL INFERENCES

From the information in the diagrams, FIGS. 1–4, the essential features of vascular development in the crown of *Rhapis* become clear. By counting

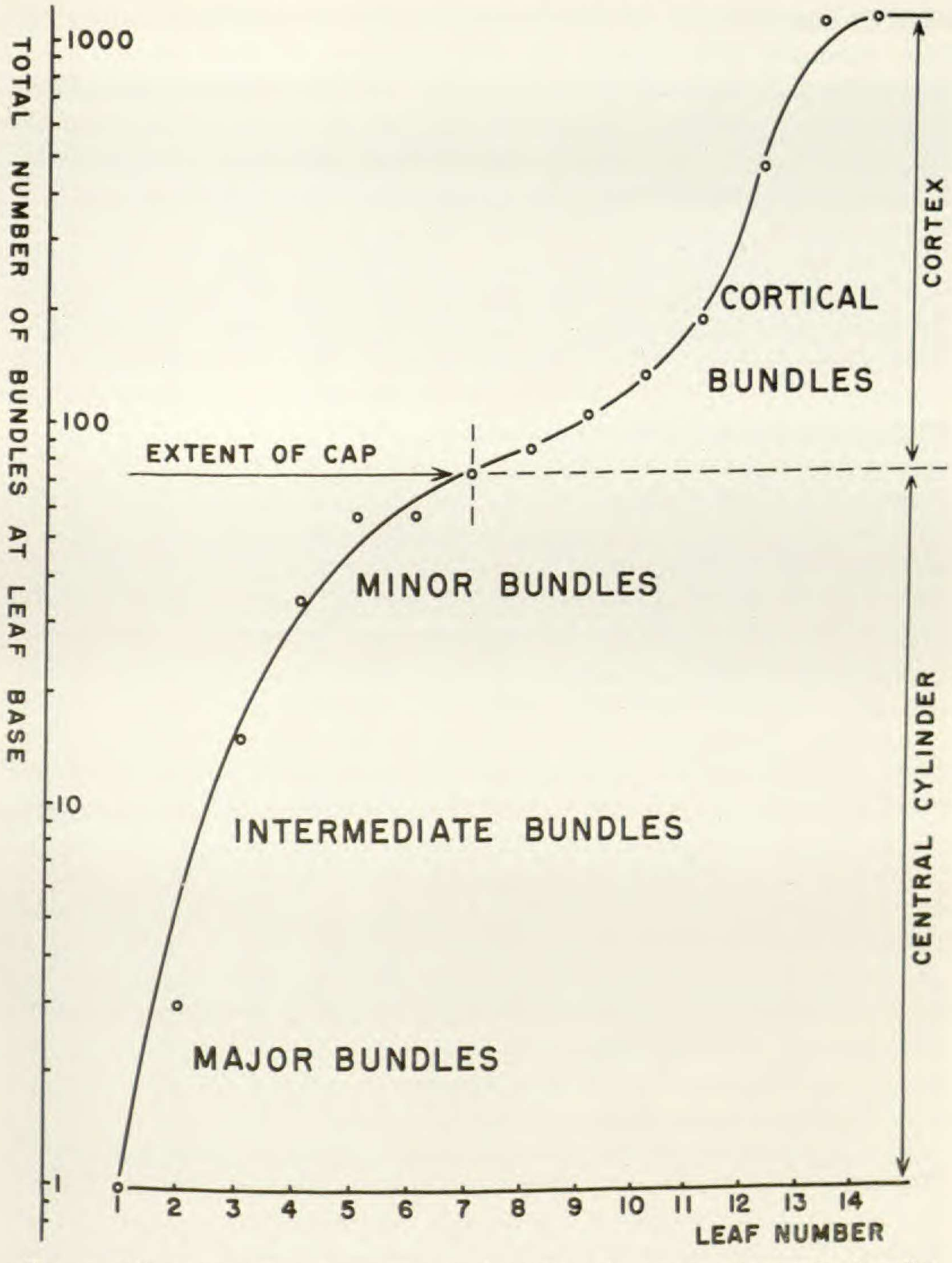


FIG. 4. *Rhapis excelsa.* Logarithmic plot of total number of vascular bundles in the base of each successively older leaf, counted in transverse sections, against leaf number. The apparent double peak of frequency of leaf trace initiation (i.e., the differential of the number of bundles) may be an artifact resulting from the difficulty of recognizing fibrous bundles in their early stage of development (see the discussion of this in the text).

the total number of recognizable bundles at the leaf insertion at each developmental stage an idea can be gained of the period over which leaf trace development continues. FIG. 4 is a plot on a logarithmic scale of such a count. It shows that each leaf produces new traces continuously for

about 13 plastochrones. In P14 the total number of bundles is established. Quite obviously major bundles are produced first, intermediate ones later, minor ones still later, and cortical ones last. Of the total number of traces in each leaf (a little over 1,000) less than 100 in the stem are bundles of the central cylinder, the remainder are cortical bundles.

Renewal of the meristematic cap in relation to the origin of different bundle categories. A consideration of successively younger leaf traces in FIG. 2 shows that vertical bundles are differentiated basipetally from the cap, or expressed more correctly, the cap grows distally from the differentiating bundles. The cap must therefore be renewed from above by tissue (below about P1–P8) which at a later stage of development differentiates as vertical-bundle branches of the leaf traces (below about P11).

As a leaf is initiated on the apical meristem its first leaf traces each make contact with the distal extremity of a vertical bundle within the cap. Because early contacts with the leaf are made in the center of the stem the upper portion of the bundle will, after differentiation, still be located in the center. As the leaf grows older newly initiated leaf traces establish vascular contact with more and more centrifugal parts of the cap. These later vascular strands are intermediate and then minor ones, they reach less far into the stem center and their leaf-contact distances are shorter. Shorter leaf-contact distances for later vascular strands are simply the result of fewer plastochrones during which the vertical bundle differentiates as a distally unconnected strand.

The rhizome crown is similar with an apparent exception that the vertical bundles in the cap keep growing even after leaf contact has been made. It must be remembered that nutrition of the rhizome crown takes place continuously from proximal regions, since scale leaves do not assimilate. In the aërial crown newly matured leaves can supply assimilates to the meristematic region (note in FIG. 1, that leaves of the developmental stage P11 and older, are green and exposed to light). Only future experimental work on the translocation of nutrients in developing regions will allow a clearer understanding of these differences.

Further mention may be made here of the relative irregularity of bundle development in the rhizome (Tomlinson & Zimmermann, 1966a). Vertical bundles occasionally (though rarely) split; vertical bundles commonly fuse to form the "long bridges" described in our earlier account. The number of vertical bundles diverging from a leaf trace is more variable than in the aërial axis and vertical bundles also seem able to continue unlimited growth in the cap without making contact distally with a leaf trace. In the mature rhizome we have followed numerous vertical bundles which had no leaf contact over distances in excess of 15 internodes. With further understanding of developmental processes in *Rhapis* it is likely that these "mistakes" can be seen to belong to the developmental norm.

Leaf trace — vertical bundle linkage and the origin of the cortex. The way in which vertical bundles maintain a constant rate of differentiation in relation to the shoot apex and the meristematic cap is

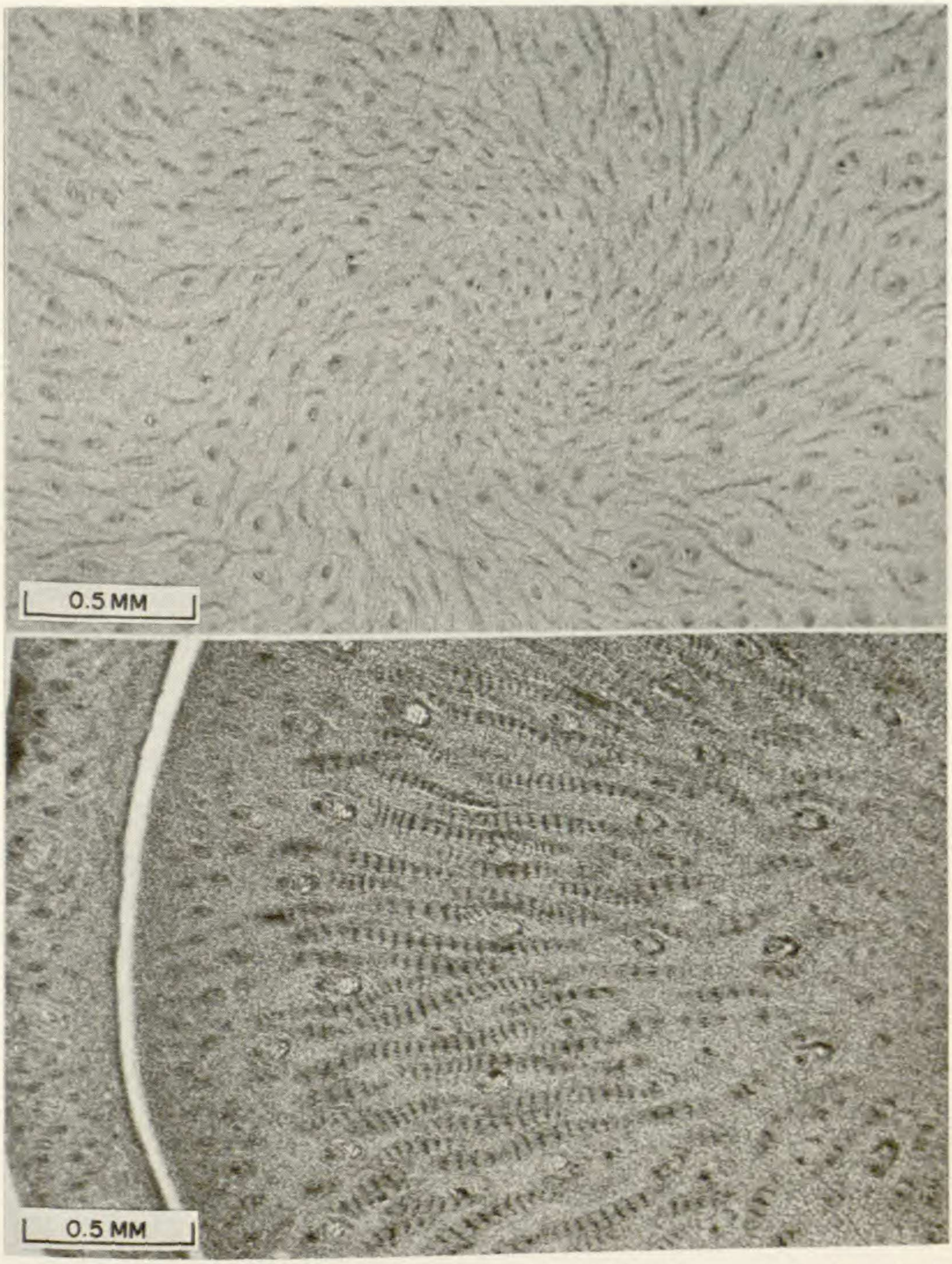


FIG. 5 (ABOVE). *Chrysalidocarpus lutescens*. Transverse section through the (bowl-shaped) crown of the stem, 0.3 millimeter below the lowest leaf-primordium insertion. The spiral path of the major leaf traces (their future helical path in the mature stem) can be seen clearly.

FIG. 6 (BELOW). *Rhapis excelsa*. Transverse section through the crown of the aërial vegetative axis 0.3 millimeter below the shoot apex proper. Leaf traces here run axially and are seen in transverse section. Vertical bundles are seen as radially-running procambial strands ("tiger tails"). These represent the peripheral crowded region of the central cylinder in which the bundles do not follow a spiral path.

evident from FIG. 2. Leaf traces are "sent out" by leaves for a period of about 13 plastochrones. The first leaf traces make contact with the central portions of the cap, subsequently initiated leaf traces contact more centrifugal parts of the cap. Still later traces "sent out" by a leaf when it is more than 7 plastochrones old appear outside the cap, i.e., outside the region of blind-ending vertical bundles. These traces must be located outside the central cylinder, that is, in the area of the stem which we recognize later as the cortex. Having failed to make contact with vertical bundles these leaf traces must end blindly below, although they may anastomose among themselves as if still "seeking" vascular contact. This blind-ending cortical system is readily observed in the cortex of both axes; it is particularly well developed in the rhizome. If we compare FIGS. 2 and 4 we can see that the cap extends radially over approximately 7 leaf insertions. Thus over a period of about 7 plastochrones vascular contact between leaf and central cylinder is possible; subsequently all leaf traces are confined to the future cortex of the mature stem.

FIGURE 4 suggests that frequency of leaf-trace initiation occurs in two peaks. This, somehow, seems to be unlikely. It could be a reflection of a

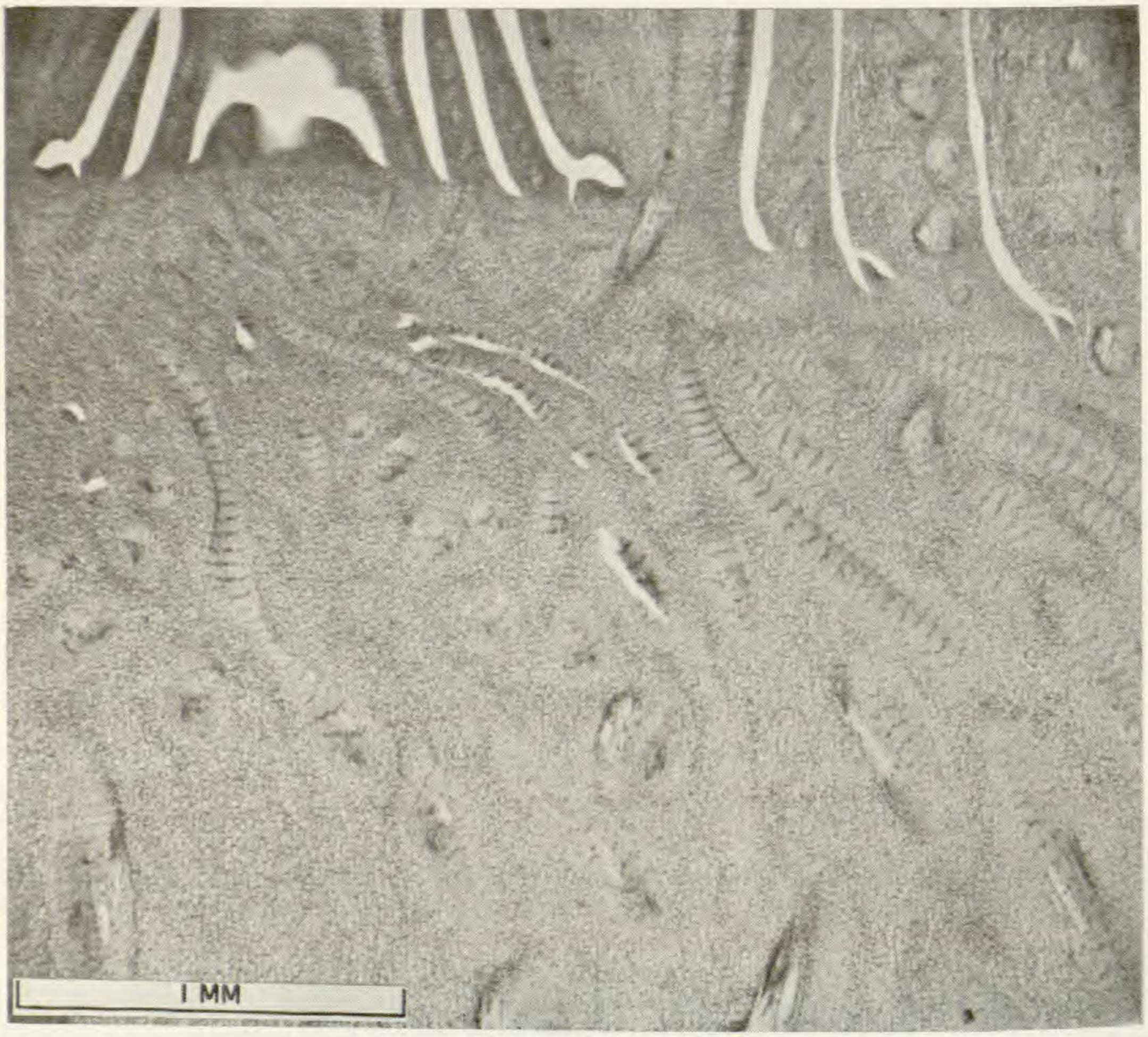


FIG. 7. *Rhapis excelsa*. Approximately median longitudinal section through the crown of the vegetative aërial axis. Because of their spiral path only short lengths of bundles can be seen. Comparison of this photograph with the diagrammatic FIG. 2 indicates major features.

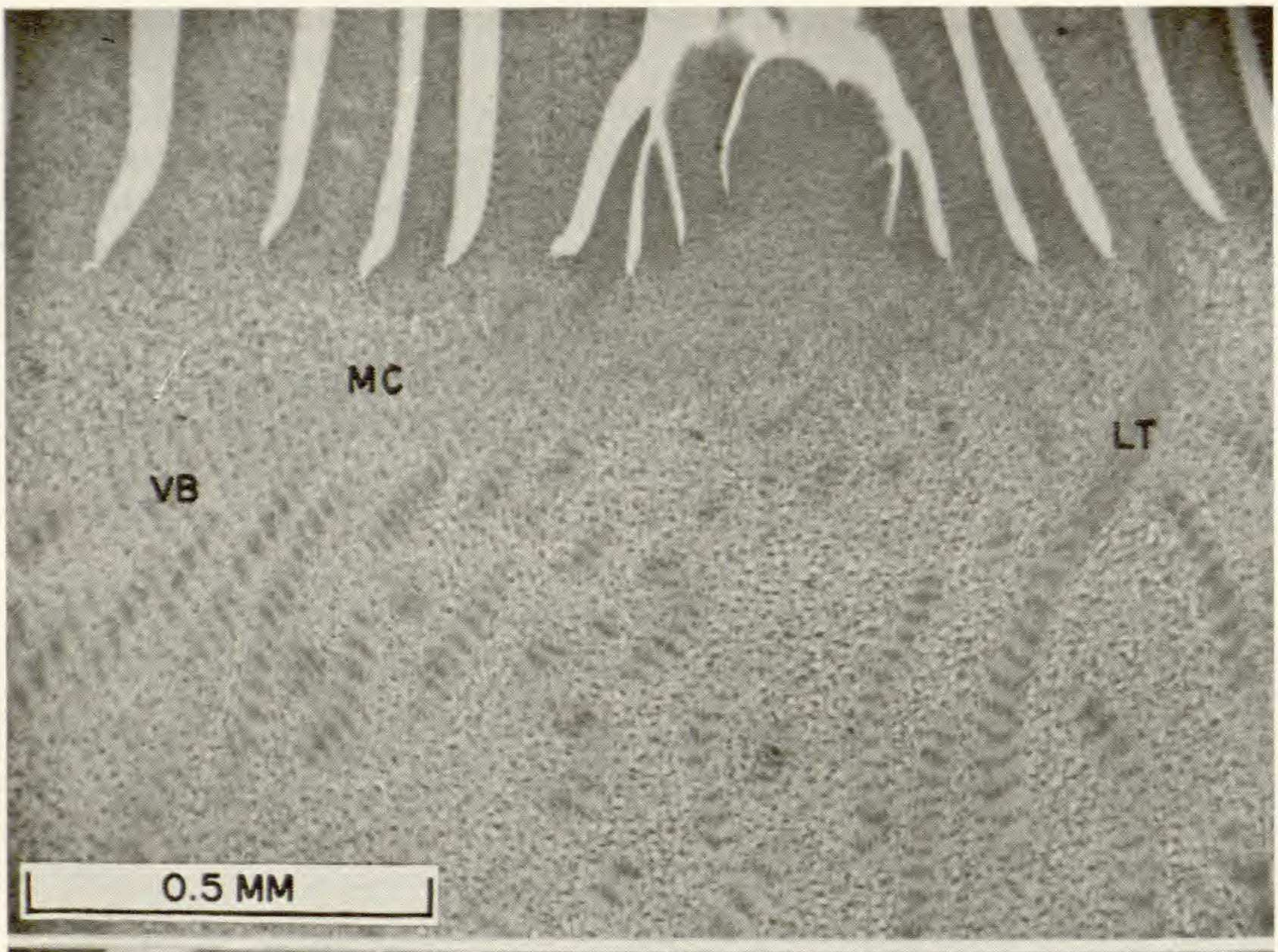
subjective phenomenon resulting from our inability to recognize early stages in differentiation of cortical compared with central bundles. In addition, the leaf base produces branching from existing leaf traces (vertical bundles, satellites, bridges) somewhat before stage 11; these are not included in the count and would "fill out" the curve. At any rate, it is reasonable to assume that only one peak of initiation frequency is involved. FIG. 4 should probably follow a simple sigmoid curve.

Thus a developmental difference between early and late differentiating bundles manifests itself in mature parts as a clear morphological boundary between central cylinder and cortex. Cortex and central cylinder both remain essentially descriptive terms; both regions are axial parts and there is no need to enter into the futile philosophical discussion as to whether cortex may or may not be regarded as a "fundamental part of the stem" or merely as a downward continuation of the leaf base, a discussion to which earlier morphologists were prone (cf. for example, Baranetzky, 1897).

Origin of vertical bundles, bridges and satellites. The question now arises as to where in development the vertical bundle diverges from the leaf trace. This can only be estimated. It is quite evident that these branches must arise in continuity with the leaf trace but directed towards the cap because no vertical bundles ending blindly below have ever been observed in the central cylinder. FIGS. 2 and 3 suggest that branching occurs when a leaf is about 11 plastochrones old in the aërial axis, 6 in the rhizome, since P11 and P6 respectively are the oldest primordia without an identifiable vertical bundle. We easily may have overlooked this vertical bundle but the configuration in FIG. 3 strongly suggests that the vertical bundle arises in continuity with the meristematic cap as soon as the leaf trace has been displaced to a position near the lower margin of the cap.

Further consideration of FIGS. 2 and 3 reveals that only the directly associated leaf can initiate development of vertical bundles, not the much higher one into which the vertical bundle ultimately proceeds. We know from our analysis of both types of axis that leaf-contact distances are of the order of a least 15 internodes for major traces. FIGS. 2 and 3 further support this as they include 14 and 13 leaves respectively but no complete leaf contact. In FIG. 3, assuming that the vertical bundle branch originates when a leaf is 6 plastochrones removed from the apex (it has already appeared in P7), then with a presumed leaf-contact distance of 15 internodes, the upper contact of this new vertical bundle would be to leaf I9,[4] a leaf which does not appear for a further 9 plastochrones. Clearly it would be absurd to assume that I9 initiates the vertical bundle in P6. Even if we were to go to the extreme of assuming that this primordium existed at the apex in an unidentifiable state, a simple computation by extrapolation from the number of cells visible in cross sections of the base of successively younger leaves gives a value for I9 of only a small fraction of a cell. It is

[4] Leaf primordia are numbered P1 (the youngest), P2, P3, etc. Non-existing ones are numbered I1 (to appear next), I2, I3, etc.



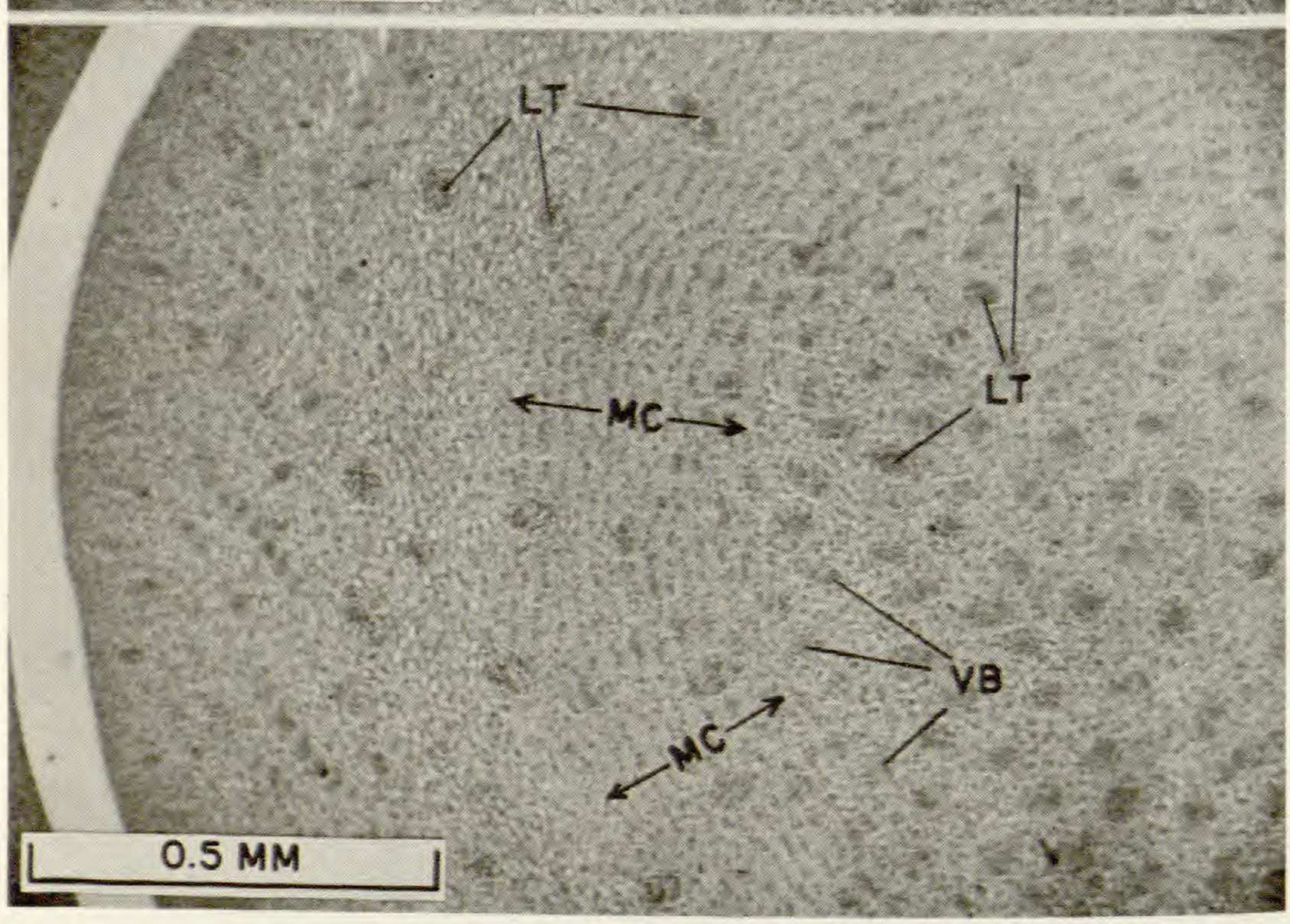


FIG. 8 (ABOVE). *Rhapis excelsa.* Approximately median longitudinal section through the crown of the rhizome. Because of their slight spiral path only short lengths of bundles can be seen. Comparison of this photograph with the diagrammatic FIG. 3 indicates major features. Procambial strands of the vertical bundles (VB) extend distally into an undifferentiated meristematic cap (MC). Leaf traces (LT).

FIG. 9 (BELOW). *Rhapis excelsa.* Transverse section through the crown of the rhizome 0.3 millimeter below the shoot apex. The undifferentiated meristematic

therefore quite impossible for vertical bundles to be initiated by non-existent leaves. This is precisely the conclusion of Priestley and his co-workers (1935) for *Alstroemeria*.

The presence of numerous bridges and satellites (Zimmermann & Tomlinson, 1965) also lends support to the idea that vertical bundles are directed apically in their development. Bridges are usually short and always attached to neighboring vertical bundles in an upward direction. We regard bridges as "frustrated" vertical bundles, i.e., both are microscopically identical and seem to be branches sent out by the leaf trace. Both can now be regarded as developmentally identical, but positional (and probably also temporal) differences determine different fates. It can be envisaged that space for continued upward development of bridges is limited (they almost invariably diverge from leaf traces more distally than vertical bundles); as a result they fuse with other, nearby existing vertical bundles. Satellites may originate in the same manner and grow towards developing inflorescences (which may later abort). The inflorescence primordium, regardless of whether it aborts later or not, may be physiologically equivalent to a shoot apex in that it "attracts" some of the branches which diverge from the leaf trace.

Concluding remarks. We may now summarize our interpretation of vascular bundle development, argued in the previous paragraphs, and the way in which we account for the overall course of bundles in *Rhapis*. First a leaf trace develops in association with a primordium into the meristematic cap. There it meets an oncoming vertical bundle. Vascular continuity thus established, bundle differentiation continues in such a way that it is displaced downward out of the cap (i.e., the cap grows upward away from it). Since the developing leaf is displaced centrifugally, i.e., in the reverse direction to which the vertical bundle had been differentiated there results a sharp bend in the bundle as a whole. This bend, the most centrally located part of the bundle, marks the point of initial contact of leaf trace with cap. Later, at a developmental stage in major traces corresponding to P10 for the aërial axis and P6 for the rhizome, branches develop away from the leaf trace towards apex and inflorescence. Of these the first formed is included in the meristematic cap as the continuing vertical bundle, later ones make bridges to existing vertical bundles while the last ones are inflorescence traces. The final configuration of leaf trace and associated branch bundles as seen in mature axes gives little indication of the direction in which these branches initially diverged, because the point of branching is displaced downward during primary thickening growth as the traces to P12, 13, and 14 suggest in FIG. 2. The sharp angles at which vertical bundles and bridges branch off the leaf trace in the mature stem is

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cap (MC) is seen as a ring penetrated by axially running leaf traces (LT). In the center of the stem (to the right of the cap in the figure and structurally proximal to the cap) are both leaf traces (LT) and vertical bundles (VB), in the periphery of the stem (to the left of the cap in the figure and morphologically distal to the cap) are only leaf traces.

incidental, for they are caused by displacement during primary thickening growth.

RHAPIS COMPARED WITH ALSTROEMERIA

Only in the work of Priestley, Scott and Gillet (1935) on *Alstroemeria* are we afforded a means of directly comparing our observations on *Rhapis* with those on another monocotyledon, although *Alstroemeria* is a great deal simpler than *Rhapis*. Nevertheless, these workers adopted an approach to a study of the developing vascular system in this non-nodal monocotyledon, similar to ours. It is noteworthy that Priestley and his associates also were forced to conclude that leaf traces in *Alstroemeria* developed acropetally long before the leaves they served became evident. On the other hand, *Alstroemeria* differs from *Rhapis* in that (1) there is no cortical system, (2) there are only 3 traces to each leaf, and (3) the leaf insertion occupies only a narrow sector of the stem circumference. This means that bundle linkage is dependent on leaf arrangement. In *Rhapis*, however, leaves encircle the stem completely at their insertion and phyllotaxis is incidental to bundle linkage. Nevertheless, a comparison of the diagrams illustrating the vascular system of *Alstroemeria* with ours for *Rhapis* suggests basic similarities in developmental terms.

SUMMARY

Vascular strands of the aërial axis and rhizome of *Rhapis excelsa* were followed in the meristematic crown with the microcinematographic method of analysis, and their course was plotted. From resulting plots vascular development is inferred. Vascular bundles of the crowded peripheral part of the central cylinder are continuous distally with a meristematic cap on the top center of which is the apical meristem proper. As each leaf develops it initiates leaf traces continuously during 14 plastochrones after inception. Leaf traces are oriented perpendicular to the vertical bundle and link with the blind ends of vertical bundles in the cap. Major vascular bundles are leaf traces initiated very early at a time when the leaf primordium is still located near the stem center. Leaf traces initiated later become intermediate and then minor bundles successively nearer the stem periphery, corresponding to the position of the leaf at the time of their initiation. The last traces, initiated outside the cap, end blindly below and differentiate into the cortical fibrous traces. The cortex, a structurally distinct region of the palm stem, is thus clearly defined by development. Determination of vascular bundles is a property of the meristematic cap effected in such a way that vascular bundles, as soon as they are continuous from a leaf primordium above to a leaf below, are displaced proximally (later centripetally) and so lost from the cap. The cap must be renewed from above by tissue which at a later stage of development differentiates as vertical-bundle branches of the leaf traces.

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